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Lifang Hou[1,2], Gabriella Andreotti[3], Andrea A. Baccarelli[4], Sharon Savage[3], Jane A. Hoppin[5], Dale P. Sandler[5], Joseph Barker[6], Zhong-Zheng Zhu[7], Mirjam Hoxha[8], Laura Dioni[8], Xiao Zhang[1], Stella Koutros[3], Laura E. Beane Freeman[3], and Michael C. Alavanja[3]

[1]Department of Preventive Medicine, and the [2]Robert H. Lurie Comprehensive Cancer Center, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA

[3]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland, USA

[4]Exposure, Epidemiology and Risk Program, Harvard School of Public Health, Boston, Massachusetts, USA

[5]Epidemiology Branch, National Institute of Environmental Health Sciences, National Institutes of Health/DHHS, Research Triangle Park, North Carolina, USA.

[6]IMS, Silver Spring, Maryland, USA

[7]Department of Oncology, No.113 Hospital of People's Liberation Army, Ningbo, China

[8]Center of Molecular and Genetic Epidemiology, Department of Environmental and Occupational Health, University of Milan and IRCCS Maggiore Policlinico Hospital, Mangiagalli and Regina Elena Foundation, Milan, Italy

Corresponding author:

Michael C.R. Alavanja, Dr.P.H.

Senior Investigator,

Division of Cancer Epidemiology and Genetics,

National Cancer Institute,

6120 Executive Blvd, Rm 8000

North Bethesda, Maryland 20892, USA

Email: alavanjm@mail.nih.gov

Telephone: 01-301-435-4720

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Abbreviations:

DDT; dichlorodiphenyltrichloroethane;

EPTC: s-ethyl dipropyl thiocarbamate;

RTL: relative telomere length;

TL: telomere length;

2,4-D: 2,4-dichlorophenoxyacetic acid;

2,4,5 T: 2,4,5 trichlorophenoxy acetic acid;

2,4,5 TP: 2 (2,4,5-trichlorophenoxy) propionic acid

Abstract

Background: Telomere length (TL) in surrogate tissues may be influenced by environmental exposures.

Objective: We aim to determine if lifetime pesticides use is associated with buccal cell TL.

Method: We examined buccal cell TL in relation to lifetime use of 48 pesticides for 1,234 cancer-free white males in the Agricultural Health Study (AHS), a prospective cohort study of 57,310 licensed pesticide applicators. Participants provided detailed information on lifetime use of 50 pesticides at enrollment (1993-7). Buccal cells were collected from 1999 to 2006. Relative telomere length (RTL) was measured using quantitative real-time PCR. Linear regression modeling evaluated the associations between specific pesticides and the logarithm of RTL, adjusting for age at buccal cell collection, state of residence, applicator license type, chewing tobacco use, and total life-time days of all pesticide use.

Results: The mean RTL for participants decreased significantly in association with increased life-time days of pesticide use for alachlor (P=0.002), 2,4-D (P=0.004), metolachlor (P=0.01), trifluralin (P=0.05), permethrin (for animal application) (P=0.02), and toxaphene (p=0.04). A similar pattern of RTL shortening was observed with the metric lifetime intensity-weighted days of pesticide use. For dichloro-diphenylchloroethane (DDT), significant RTL shortening was observed for lifetime intensity-weighted days (P=0.04), but not for lifetime days of DDT use (P=0.08). No significant RTL lengthening was observed for any pesticide.

Conclusion: Seven pesticides previously associated with cancer risk in the epidemiologic literature were inversely associated with RTL in buccal cell DNA among cancer-free pesticide applicators. Replication of these findings is needed since we cannot rule out chance or fully rule out bias.

Introduction

Pesticides are widely used in the US and worldwide and are pervasive in our environment. Several pesticides have been associated with various cancers in experimental studies [summarized by the USEPA (EPA 2012)] and in epidemiologic studies of farmers (Blair and Freeman 2009) and pesticide manufacturing workers (Acquavella et al. 1996; Fryzek et al. 1997; Kogevinas et al. 1997; Leet et al. 1996), and among register pesticide applicators, including the Agricultural Health Study (AHS) cohort, one of the largest prospective studies of pesticide applicators (Alavanja et al. 1996).

The mechanisms by which pesticides may be linked to cancers in humans are unclear. Potential mechanisms include oxidative stress, DNA damage, chromosome aberration, immune response abnormality, and chronic inflammation (Casale et al. 1998; Hooghe et al. 2000; Stiller-Winkler et al. 1999; Undeger and Basaran 2005). These biological processes are also involved in telomere shortening (von Zglinicki 2002). The genetic integrity of the genome is maintained, in part, by the architecture of telomeres (Artandi et al. 2000; Meeker et al. 2004). Telomeres typically shorten with each cell division. When telomeres reach a critical length, cellular apoptosis or senescence is triggered. Cancer cells bypass these pathways and continue to divide despite the presence of chromosomal instability (Blasco 2005). Human epidemiologic investigations, mostly from case-control studies, have suggested that telomere length (TL) in surrogate tissues (*i.e.,* blood or buccal cells) is associated with some but not all cancers (Broberg et al. 2005; Hou et al. 2009; Jang et al. 2008; McGrath et al. 2007; Risques et al. 2007; Shao et al. 2007; Widmann et al. 2007). While most studies have reported that shorter telomeres in surrogate tissue are positively associated with cancer (as reviewed in Ma et al. 2011; Wentzensen

et al, 2011), longer telomeres have been associated with cancer in some studies and reviews (Gramatges et al. 2010; Han et al. 2009; Lan et al. 2009; Shen et al. 2011; Svenson et al. 2009).

The potential effects of pesticide exposure on TL in surrogate tissues have not been well characterized, although associations with telomere shortening have been reported for occupational exposures (Eshkoor et al. 2011) and persistent organic pollutants (Shin et al.2010). In the present study, we examine whether lifetime use of 48 pesticides is associated with telomere shortening in buccal cell DNA from 1,234 white male cancer-free licensed pesticide applicators participating in the AHS.

Materials and Methods

Study population: A detailed description of the AHS has been published (Alavanja et al. 1996). Briefly, 57,310, or 82%, of pesticide applicators seeking pesticide licensing in Iowa and North Carolina were enrolled between December 13, 1993 and December 31, 1997. All data used in these analyses were based on AHS data releases P1REL0506.01. Enrolled participants were licensed private pesticide applicators (mostly farmers) residing in Iowa and North Carolina, and commercial applicators residing in Iowa. All pesticide applicators completed an enrollment questionnaire that inquired about ever/never use of 50 pesticides (NIH, National Institutes of Health: http://aghealth.nci.nih.gov/questionnaires.html), as well as the duration (years) and frequency (average days/year) of use for 22 of these pesticides. In addition, 44% completed a second take-home questionnaire at enrollment that inquired about the duration and frequency of use of the remaining 28 pesticides. Approximately five years after enrollment, participants completed a follow-up phone interview to collect additional pesticide use and medical history information and were asked to provide a mouthwash rinse sample for extraction of DNA from

buccal cells. Participants who agreed to provide buccal cells were sent a buccal cell collection kit and a postage-paid, padded envelope. Informed consent for buccal cell collection and buccal cell analyses associated with potential carcinogenic risk assessment was obtained at the time of collection and the study protocol and informed consent was reviewed by all relevant Institutional Review Boards. 36,342 (63%) participants completed the follow-up interview and 20,421 (56%) returned buccal cells. No meaningful difference was observed between those who donated buccal cells and those that did not (Engel et al. 2002).

As part of a nested case-control study of prostate cancer, 1,372 participants who had no history of prostate cancer (controls) and were over the age of 40 years at the time of buccal cell collection were initially selected for telomere length measurement. Of these, 1,234 met inclusion criteria for the telomere length analysis. Participants excluded from the analysis were 115 men diagnosed with any cancer prior to buccal cell collection or within 3 years after buccal cell sampling, 14 men who donated a buccal cell sample but did not provide a completed written consent at the time of the present analysis, and 9 men who were non-white. The present analysis was limited to white applicators because of the small numbers of non-white applicators in the AHS cohort (Alavanja et al. 1996).

Pesticide Exposure: Two pesticide exposure metrics were used. Pesticide use was evaluated as both lifetime days of pesticide use (years of use x days per year) and lifetime intensity-weighted days of pesticide use (lifetime exposure days x intensity score). The intensity score was computed from an algorithm that took into account exposure-modifying factors such as application method and protective equipment use (Coble et al. 2011).

Buccal cell collection: Buccal cells were collected from 1999 through 2006 using a mouthwash "swish and spit" collection technique (Garcia-Closas et al. 2001). Buccal cells collection vials were returned to the NCI repository via a postage-paid, padded envelope. All samples were stored at the NCI repository at -80 °C. DNA from buccal cells was extracted using the Wizard® Genomic DNA Purification Kit (Promega Corp., Madison, WI).

Telomere length measurements: Relative telomere length (RTL) in buccal DNA was measured at the Laboratory of Environmental Epigenetics, Center of Molecular and Genetic Epidemiology, Milan University, Italy, using quantitative real-time PCR as described previously (Cawthon 2002). Briefly, this method measures RTL in genomic DNA by determining the ratio of telomere repeat copy number (T) to single copy gene (S) (*36B4* gene located on chromosome 12, which encodes acidic ribosomal phosphoprotein PO) copy number (T/S ratio) in individual samples relative to a reference pooled DNA (Boulay et al. 1999). The reference pooled DNA was created using samples from 60 participants randomly selected from the population sample selected for this study, and used to generate a fresh standard curve, ranging from 0.25 to 8 ng/µl in every T and S PCR run (Supplemental Material, Figure S1). All samples were successfully run in duplicate with a 100% completion rate. The inter-batch variability [coefficient of variation (CV)] in this study was 8.1%. The primer sequences and concentrations were: GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT (270 nM) and TCCCGACTATCCCTATCCCTATCCCTATCCCTATCC-CTA (900 nM) for telomere; and CAGCAAGTGGGAAGGTGTAATCC (300 nM) and CCCATTCTATCATCAACGGGTACAA (500 nM).

The T (telomere) PCR mix was: iQ SYBR Green Supermix (Bio-Rad) 1×, tel1b 100 nM, tel2b 900 nM, DMSO 1%, EDTA 1×. The S (human beta-globin) PCR mix was: iQ SYBR Green

Supermix (Bio-Rad) 1×, hbg1 300 nM, hbg2 700 nM, DMSO 1%, DTT 2,5 mM, EDTA 1×. We used the PCR primer sets previously described by McGrath et al. (2007). We used pooled DNA from 20 referents (500 ng for each sample), randomly selected from samples of this same study, to create a fresh standard curve, ranging from 8 ng/µl to 0,5 ng/µl, at every T and S PCR run. All samples contained E. coli DNA heated at 96°C × 10 minutes and cooled at room temperature. 15 ng of DNA samples was added to each reaction (final volume 20 µl). All PCRs were performed on a DNA Engine thermal cycler Chromo4 (Bio-Rad, Hercules, California, USA). The thermal cycling profile for both amplicons started with a 95°C incubation for 3 minutes to activate the hot-start iTaq DNA polymerase. The T PCR continued with 25 cycles at 95°C for 15s, and anneal/extend at 54°C for 49s. The S PCR continued with 35 cycles at 95°C for 15s, anneal at 58°C for 1s, extend at 72°C for 15s. At the end of each reaction, a melting curve was used for both T and S PCRs. All samples were run in triplicates.

Statistical analysis: The means of all three RTL measurements were used in statistical analyses. Mean RTL values reported in Table 1 are the arithmetic mean stratum specific estimates of RTL along with the standard deviation (SD) of these values. Because RTL and both pesticide exposure metrics (i.e., lifetime-days and lifetime intensity-weighted days) had right-skewed frequency distributions, a natural logarithm transformation was applied to RTL and both exposure metrics. Linear regression models were used to estimate the change in RTL with increasing pesticide use on a continuous scale (i.e. lifetime-days and lifetime intensity-weighted days). For presentation purposes, we also calculated the mean RTL for the reference group (no use of the pesticide) and each tertile of lifetime intensity-weighted days of pesticide use; however, the continuous measure was used in the analysis for testing statistical significance. Two pesticides (trichlorofon and ziram) had small numbers of exposed participants (n<20) and

were dropped from the analyses. P-values for the linear regression coefficient between each pesticide and RTL, showing the continuous change in RTL with increasing days of use, were adjusted for age at buccal cell sample collection (as a continuous variable), state of residence (IA vs. NC), applicator license type (private, commercial), use of chewing tobacco regularly for six months or longer (yes vs. no) and total lifetime-days of all pesticide use (continuous). Further adjusting the linear regression model for BMI (continuous), alcohol consumption (none vs. any; or none, <3, or ≥ 3 drinks per week), smoking (pack years, current vs. never, ever vs. never), and self-reported cardiovascular disease, diabetes, and high blood pressure produced comparable results (data not shown), and these factors were not included in our final models. All tests were two-sided and $P \leq 0.05$ was considered significant. All statistical analyses were conducted using AHS Data Release version P1REL0506.01 and 9.2 (SAS, Cary, N.C.).

Results

Regression analysis p values for model coefficients of ln-transformed pesticide exposures adjusted for age at buccal cell collection are shown according to selected characteristics in Table 1. RTL was negatively associated with age at buccal cell sample collection (p=0.003). Commercial pesticide applicators had shorter RTL (mean = 1.08) than private applicators (mean = 1.21, p=0.01). The mean RTL in Iowa applicators (1.19) was significantly shorter than the mean value in North Carolina applicators (mean = 1.24, p =0.03). The mean RTL was significantly longer for men who used chewing tobacco for six months or longer (mean = 1.27) than men who did not (mean = 1.19, p =0.01). Self-reported education, BMI, smoking status, pack-years smoked, alcohol consumption amount, family history of any cancers, cardiovascular diseases, diabetes, and blood pressure were not significantly associated with RTL. It is worth

noting that although RTL among current smokers is not statistically significant (overall p>0.05), both current smokers and those chewing tobacco had longer telomeres. All study participants applied some pesticides, but a comparison of those with more than the median number of applications days of total pesticide use vs. those with less than the median number did not show any difference in RTL.

Among the 48 pesticides examined, increasing lifetime-days of pesticide use for six pesticides (alachlor, metolachlor, trifluralin, 2,4-D, permethrin, and toxaphene) were significantly ($p<0.05$) associated with decreases in RTL after adjustment for age at buccal cell collection, state of residence, license type, use of chewing tobacco, and total pesticide-application days (Table 2; see Supplemental Material, Table S1 for results for the other pesticides evaluated). Of these, four were herbicides: alachlor (P=0.002); metolachlor (P=0.01); trifluralin (P=0.05) and 2,4-D (P=0.004), and two were insecticides: permethrin use (P=0.02) and toxaphene (P=0.04). No significant RTL lengthening was observed for any pesticide (Table 2 and Supplemental Material, Table S1).

Comparable results were observed with lifetime intensity-weighted days of pesticide use (Table 2) for alachlor (P=0.005), metolachlor (P=0.01), permethrin (P=0.02), 2,4-D (P=0.02), and toxaphene (P=0.05). RTL also decreased with increasing use of trifluralin (P=0.06), but the association was not statistically significant. A statistically significant negative association with RTL was estimated for intensity-weighted lifetime-days of DDT use (P=0.03), but not for lifetime-days of DDT use (P=0.08). Adjustment for total days of pesticide use and for use of chewing tobacco did not affect the associations with pesticides in any meaningful way.

Discussion

In this study, seven pesticides were negatively associated with RTL in buccal cell DNA among cancer-free pesticide applicators over the age of 40. Increasing lifetime-days of use of six pesticides used in agriculture, (alachlor, metolachlor, trifluralin, permethrin, 2, 4-D, and toxaphene), were associated with significantly shorter telomeres after controlling for age at buccal cell collection, state of residence, license type, use of chewing tobacco, and total pesticide use days. Associations were similar between lifetime-days and intensity-weighted days of use of alachlor, metolachlor, permethrin, 2,4-D, and toxaphene. RTL also decreased with increasing lifetime intensity-weighted days of use of trifluralin, but the association was not statistically significant. For DDT, RTL shortening was significant in association with lifetime intensity-weighted days, but not lifetime-days of use.

The only study reporting data on pesticides and telomere length found that high levels of exposure to persistent organic pollutants (POPs), including organochlorine (OC) pesticides, polychlorinated biphenyls (PCBs), and polybrominated diphenylethers (PBDEs) were associated with decreased RTL in peripheral blood leukocytes DNA, but low levels of exposure were associated with increased RTL in an apparently healthy Korean population (Shin et al. 2010). However, due to the limited sample size, and very small numbers of observations at higher levels of exposure, more studies are needed to confirm the observation.

We observed that use of seven pesticides, as estimated by one or more exposure metrics, was associated with significantly decreasing RTL. Pesticide use has been noted to cause oxidative stress in humans (Honda et al. 2001; Houben et al. 2008; Meeker et al. 2004; von Zglinicki 2002), and telomere shortening has been associated with cumulative oxidative stress (Houben et

al. 2008; von Zglinicki 2002). Telomeres are remarkably sensitive to damage by oxidative stress, because of the high guanine content in specific telomere sequences and the deficiency in the repair of single-strand breaks (Honda et al. 2001; Meeker et al. 2004; von Zglinicki 2002). Pesticide exposure may also lead to telomere shortening by causing inflammation. Figgs et al., reported that urinary 2,4-D concentration was associated with an increased lymphocyte replicative index, a cell proliferation biomarker (Figgs et al. 2000). The replicative index for lymphocytes was higher among applicators than non-applicators and higher among applicators after spraying than before spraying. Telomeric DNA is dynamic, and TL typically shortens with each cell division (Hou et al. 2012). Therefore, the increased lymphocytes replicative index associated with the use of 2,4-D could be associated with inflammation since extensive cell proliferation and clonal expansion is an essential part of an inflammatory response (Hodes et al. 2002).

Of the seven pesticides associated with telomere shortening, four are herbicides and three are insecticides. The use of these chemicals was not strongly correlated with each other in our sample (the range of correlation coefficients between the seven pesticides varied from 0.1 to 0.24). These pesticides belong to different chemical classes and there were no chemical or functional classes where all pesticides were linked to RTL shortening.

Alachlor has been shown to induce chromosomal aberrations in mice bone marrow cells (Meisner LF et al. 1992) and causes chromosomal damage in *in vitro* experimental studies using Chinese hamster ovary cells (Lin et al. 1987), which may be related to TL shortening. In the AHS, a positive association between alachlor and the incidence of lymphohematopoietic cancers was found among applicators (Lee et al. 2004a), but no excess cancer risk was observed in a study of alachlor manufacturing workers (Acquavella et al. 2004).

Metolachlor has been associated with lung cancer in the AHS (Alavanja et al. 2004) and the USEPA classifies metolachlor as a 'possible human carcinogen' (EPA group C) (EPA 1995). Trifluralin has been associated with colon cancer in the AHS (Kang et al. 2008) and the USEPA classifies trifluralin as a 'possible human carcinogen' (EPA group C) (EPA 1996). 2,4-D has been associated with prostate cancer in a large case-control study in British Columbia (Band et al. 2011) but not in the AHS cohort (Koutros et al. 2012), and with NHL in a number of studies (Burns et al. 2011; Hoar et al. 1986; McDuffie et al. 2001; Miligi et al. 2006). The USEPA classifies 2,4-D as 'not classifiable' (EPA Group D) with regard to human carcinogenicity (EPA 2005b), while the International Agency for Research on Cancer (IARC) classifies the chlorphenoxy herbicide group as possibly carcinogenic to humans (IARC group 2B) (IARC 2001). Permethrin has been associated with multiple myeloma in the AHS (Rusiecki et al. 2009). In laboratory studies, treatment with a high dose of permethrin induced significant lymphocyte DNA damage in a rat model (Gabbianelli et al. 2004). DDT and toxaphene are considered 'possible human carcinogens' (IARC group 2B) by the IARC (IARC 2001). In a recent Danish study, conducted within a prospective cohort, prediagnostic adipose concentrations of DDT demonstrated a significant positive monotonic dose-response trend with NHL incidence (Brauner et al. 2012).

This investigation is unique in that we have a relatively large population of licensed pesticide applicators who provided reliable information regarding their pesticide application history (Blair et al. 2002; Coble et al. 2011). In this study, we were able to examine the relationship between cumulative lifetime use of specific pesticides and RTL in a cancer-free population (i.e., study participants did not have a cancer diagnoses). Furthermore, we were able to adjust for potential confounding factors related to RTL. In the AHS, *a priori* derived algorithm scores that

incorporated several exposure determinants were used to predict pesticide exposure intensity. These algorithm scores have been shown to predict urinary pesticides levels (Thomas et al. 2010, Coble 2011). The significant decrease in RTL with age that we observed was expected (Blasco 2005) based on the literature, and adds confidence to our pesticide-related findings. Most pesticides used by applicators are mixtures of one active ingredient and several 'inerts' that are not pesticides, and there are some products that contain more than one active ingredient. The co-occurrence of active ingredients is an intractable problem for an epidemiological study assessing a lifelong occupational history of pesticide use. This problem, however, is less common than the concern we are able to address, which is that over a lifetime applicators may use more than one product for the same or different crops. In this study we controlled for the total pesticide-exposure days, and found no meaningful change in the associations, though confounding by specific pesticides cannot be ruled out. While potential confounding from other occupational exposures is possible, the magnitude of bias due to this confounding was reported to be minimal in the AHS and not likely to be associated with pesticide exposures (Coble et al. 2002) . The fact the other life-style factors (e.g., smoking and alcohol drinking) and chronic health conditions (e.g., cardiovascular disease, high blood pressure) were not significantly associated with telomere length in our population may be a result of our population being more physically active and older than some previous studies. Physical activity has been shown to increase TL (Du et al., 2012) and TL seems to be less influenced by lifestyle factors in older populations (Tianinen et al., 2012). Future studies with increased statistical power will be needed to assess this issue more completely.

In this study, we used buccal cell DNA for TL measurement collected at a single point in time after exposure assessment was made. Buccal cells can be easily and inexpensively collected and

are therefore a convenient resource to evaluate TL-related disease risks. Future studies may benefit from collecting buccal cells at multiple points in time to assess the rate at which telomeres are shortened. Our results should, however, be interpreted with caution since TL in buccal DNA may be different from that in diseased tissue or blood DNA (Prescott et al. 2012; Wong et al. 2011). We observed that cumulative lifetime use of some pesticides is associated with telomere shortening in buccal cell DNA, although we cannot rule out the possibility of bias due to uncontrolled confounding, or associations due to chance because of the multiple comparisons that were made. These findings suggest that specific pesticides may contribute to telomere shortening and may serve as a mechanism for development of some diseases. Given that this is one of the first studies of pesticide exposure and telomere length, we view the work as hypothesis generating rather than hypothesis testing.

References

Acquavella JF, Delzell E, Cheng H, Lynch CF, Johnson G. 2004. Mortality and cancer incidence among alachlor manufacturing workers 1968-99. Occup Environ Med 61(8): 680-685.

Acquavella JF, Riordan SG, Anne M, Lynch CF, Collins JJ, Ireland BK, et al. 1996. Evaluation of mortality and cancer incidence among alachlor manufacturing workers. Environ Health Perspect 104(7): 728-733.

Alavanja MC, Sandler DP, McMaster SB, Zahm SH, McDonnell CJ, Lynch CF, et al. 1996. The Agricultural Health Study. Environ Health Perspect 104(4): 362-369.

Artandi SE, Chang S, Lee SL, Alson S, Gottlieb GJ, Chin L, et al. 2000. Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. Nature 406(6796): 641-645.

Band PR, Abanto Z, Bert J, Lang B, Fang R, Gallagher RP, et al. 2011. Prostate cancer risk and exposure to pesticides in British Columbia farmers. Prostate 71(2): 168-183.

Blair A, Freeman LB. 2009. Epidemiologic studies in agricultural populations: observations and future directions. J Agromedicine 14(2): 125-131.

Blair A, Tarone R, Sandler D, Lynch CF, Rowland A, Wintersteen W, et al. 2002. Reliability of reporting on life-style and agricultural factors by a sample of participants in the Agricultural Health Study from Iowa. Epidemiology 13(1): 94-99.

Blasco MA. 2005. Telomeres and human disease: ageing, cancer and beyond. Nat Rev Genet 6(8): 611-622.

Boulay JL, Reuter J, Ritschard R, Terracciano L, Herrmann R, Rochlitz C. (1999) Gene dosage by quantitative real-time PCR. Biotechniques, 27, 228–230, 232.

Brauner EV, Sorensen M, Gaudreau E, LeBlanc A, Eriksen KT, Tjonneland A, et al. 2012. A prospective study of organochlorines in adipose tissue and risk of nonHodgkin lymphoma. Environ Health Perspect 120(1): 105-111.

Broberg K, Bjork J, Paulsson K, Hoglund M, Albin M. 2005. Constitutional short telomeres are strong genetic susceptibility markers for bladder cancer. Carcinogenesis 26(7): 1263-1271.

Burns C, Bodner K, Swaen G, Collins J, Beard K, Lee M. 2011. Cancer incidence of 2,4-D production workers. Int J Environ Res Public Health 8(9): 3579-3590.

Casale GP, Scott DM, Anderson JR, Vitzthum EF, Gold RE. 1998. A preliminary study of immunologic and hematologic profiles of peripheral blood from Nebraska farmers who apply pesticides to their fields. J Toxicol Clin Toxicol 36(3): 183-194.

Cawthon RM. 2002. Telomere measurement by quantitative PCR. Nucleic Acids Res 30(10): e47.

Coble J, Hoppin JA, Engel L, Elci OC, Dosemeci M, Lynch CF, et al. 2002. Prevalence of exposure to solvents, metals, grain dust, and other hazards among farmers in the Agricultural Health Study. J Expo Anal Environ Epidemiol 12(6): 418-426.

Coble J, Thomas KW, Hines CJ, Hoppin JA, Dosemeci M, Curwin B, et al. 2011. An updated algorithm for estimation of pesticide exposure intensity in the agricultural health study. Int J Environ Res Public Health 8(12): 4608-4622.

Du M, Prescott J, Kraft P, Han J, Giovannucci E, Hankinson SE, De Vivo I. 2012. Physical activity, sedentary behavior and leukocyte telomere length in women. Am J Epidemiol 175 (5): 414-422.

Engel LS, Rothman N, Knott C, Lynch CF, Logsden-Sackett N, Tarone RE, et al. 2002. Factors associated with refusal to provide a buccal cell sample in the Agricultural Health Study. Cancer Epidemiol Biomarkers Prev 11(5): 493-496.

EPA. 1995. US Environmental Protection Agency. Registration Eligibility Decision (RED) for Metolachlor. EPA 738-R-95-006 http://www.epa.gov/oppsrrd./REDs/0001.pdf

EPA. 1996. US Environmental Protection Agency. Registration Eligibility Decision (RED) for Trifluralin http://www.epa/oppsrrd/REDs/0179.pdf

EPA. 2005a. US Environmental Protection Agency. Guidelines for Carcinogenic. March 2005. EPA/630/P-03/001F. Washington, D.C. www.epa.gov/cancerguidelines/

EPA. 2005b. US Environmental Protection Agency. Registration Eligibility Decision (RED):2,4-D: EPA 738-R-05-002. http://www.epa.gov/oppsrrd1/REDs/24d_red.pdf

Eshkoor SA, Ismail P, Rahman SA, Moin S. 2011. Does GSTP1 polymorphism contribute to genetic damage caused by ageing and occupational exposure? Arh Hig Rada Toksikol 62(4): 291-298.

Figgs LW, Holland NT, Rothmann N, Zahm SH, Tarone RE, Hill R, et al. 2000. Increased lymphocyte replicative index following 2,4-dichlorophenoxyacetic acid herbicide exposure. Cancer Causes Control 11(4): 373-380.

Fryzek JP, Garabrant DH, Harlow SD, Severson RK, Gillespie BW, Schenk M, et al. 1997. A case-control study of self-reported exposures to pesticides and pancreas cancer in southeastern Michigan. Int J Cancer 72(1): 62-67.

Gabbianelli R, Nasuti C, Falcioni G, Cantalamessa F. 2004. Lymphocyte DNA damage in rats exposed to pyrethroids: effect of supplementation with Vitamins E and C. Toxicology 203(1-3): 17-26.

Garcia-Closas M, Egan KM, Abruzzo J, Newcomb PA, Titus-Ernstoff L, Franklin T, et al. 2001. Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. Cancer Epidemiol Biomarkers Prev 10(6): 687-696.

Gramatges MM, Telli ML, Balise R, Ford JM. 2010. Longer relative telomere length in blood from women with sporadic and familial breast cancer compared with healthy controls. Cancer Epidemiol Biomarkers Prev 19(2): 605-613.

Han J, Qureshi AA, Prescott J, Guo Q, Ye L, Hunter DJ, et al. 2009. A prospective study of telomere length and the risk of skin cancer. J Invest Dermatol 129(2): 415-421.

Hoar SK, Blair A, Holmes FF, Boysen CD, Robel RJ, Hoover R, et al. 1986. Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. JAMA 256(9): 1141-1147.

Hodes RJ, Hathcock KS, Weng NP. 2002. Telomeres in T and B cells. Nat Rev Immunol 2(9): 699-706.

Honda S, Hjelmeland LM, Handa JT. 2001. Oxidative stress--induced single-strand breaks in chromosomal telomeres of human retinal pigment epithelial cells in vitro. Invest Ophthalmol Vis Sci 42(9): 2139-2144.

Hooghe RJ, Devos S, Hooghe-Peters EL. 2000. Effects of selected herbicides on cytokine production in vitro. Life Sci 66(26): 2519-2525.

Hou L, Zhang X, Gawron AJ, Liu J. 2012. Surrogate tissue telomere length and cancer risk: shorter or longer? Cancer Lett 319(2): 130-135.

Hou L, Savage SA, Blaser MJ, Perez-Perez G, Hoxha M, Dioni L, et al. 2009. Telomere length in peripheral leukocyte DNA and gastric cancer risk. Cancer Epidemiol Biomarkers Prev 18(11): 3103-3109.

Houben JM, Moonen HJ, van Schooten FJ, Hageman GJ. 2008. Telomere length assessment: biomarker of chronic oxidative stress? Free Radic Biol Med 44(3): 235-246.

IARC. 2001. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Some thyrotropic Agents. .

Jang JS, Choi YY, Lee WK, Choi JE, Cha SI, Kim YJ, et al. 2008. Telomere length and the risk of lung cancer. Cancer Sci 99(7): 1385-1389.

Kang D, Park SK, Beane-Freeman L, Lynch CF, Knott CE, Sandler DP, et al. 2008. Cancer incidence among pesticide applicators exposed to trifluralin in the Agricultural Health Study. Environ Res 107(2): 271-276.

Kogevinas M, Becher H, Benn T, Bertazzi PA, Boffetta P, Bueno-de-Mesquita HB, et al. 1997. Cancer mortality in workers exposed to phenoxy herbicides, chlorophenols, and dioxins. An expanded and updated international cohort study. Am J Epidemiol 145(12): 1061-1075.

Koutros S, Beane Freeman LE, Lubin JH, Heltsch SL, Andreotti G, Barry KH, Della Valle CT, Hoppin JA, Sandler DP, Lynch CF, Blair A, Alavanja MCR. 2012. Risk of total aggressive prostate cancer and pesticide use in the Agricultural Health Study, Am J Epidemiol [Epub ahead of print, Nov. 21].

Lan Q, Cawthon R, Shen M, Weinstein SJ, Virtamo J, Lim U, et al. 2009. A prospective study of telomere length measured by monochrome multiplex quantitative PCR and risk of non-Hodgkin lymphoma. Clin Cancer Res 15(23): 7429-7433.

Lee WJ, Hoppin JA, Blair A, Lubin JH, Dosemeci M, Sandler DP, et al. 2004a. Cancer incidence among pesticide applicators exposed to alachlor in the Agricultural Health Study. Am J Epidemiol 159(4): 373-380.

Leet T, Acquavella J, Lynch C, Anne M, Weiss NS, Vaughan T, et al. 1996. Cancer incidence among alachlor manufacturing workers. Am J Ind Med 30(3): 300-306.

Lin MF, Wu CL, Wang TC. 1987. Pesticide clastogenicity in Chinese hamster ovary cells. Mutat Res 188(3): 241-250.

Ma H, Zhou Z, Wei S, Liu Z, Pooley KA, Dunning AM, et al. 2011. Shortened telomere length is associated with increased risk of cancer: a meta-analysis. PLoS One 6(6): e20466.

McDuffie HH, Pahwa P, McLaughlin JR, Spinelli JJ, Fincham S, Dosman JA, et al. 2001. Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross-Canada study of pesticides and health. Cancer Epidemiol Biomarkers Prev 10(11): 1155-1163.

McGrath M, Wong JY, Michaud D, Hunter DJ, De Vivo I. 2007. Telomere length, cigarette smoking, and bladder cancer risk in men and women. Cancer Epidemiol Biomarkers Prev 16(4): 815-819.

Meeker AK, Hicks JL, Iacobuzio-Donahue CA, Montgomery EA, Westra WH, Chan TY, et al. 2004. Telomere length abnormalities occur early in the initiation of epithelial carcinogenesis. Clin Cancer Res 10(10): 3317-3326.

Meisner LF, Belluck DA, Roloff BD. 1992. Cytogenetic effects of alachlor and/or atrazine in vivo and in vitro. Environ Mol Mutagen 19(1): 77-82.

Miligi L, Costantini AS, Veraldi A, Benvenuti A, Vineis P. 2006. Cancer and pesticides: an overview and some results of the Italian multicenter case-control study on hematolymphopoietic malignancies. Ann N Y Acad Sci 1076: 366-377.

NIH (National Institutes of Health). Agricultural Health Study Questionnaires. http://aghealth.nci.nih.gov/questionnaires.html (accessed 30 May 2013).

Prescott J, Wentzensen IM, Savage SA, De Vivo I. 2012. Epidemiologic evidence for a role of telomere dysfunction in cancer etiology. Mutat Res 730(1-2): 75-84.

Risques RA, Vaughan TL, Li X, Odze RD, Blount PL, Ayub K, et al. 2007. Leukocyte telomere length predicts cancer risk in Barrett's esophagus. Cancer Epidemiol Biomarkers Prev 16(12): 2649-2655.

Shao L, Wood CG, Zhang D, Tannir NM, Matin S, Dinney CP, et al. 2007. Telomere dysfunction in peripheral lymphocytes as a potential predisposition factor for renal cancer. J Urol 178(4 Pt 1): 1492-1496.

Shen M, Cawthon R, Rothman N, Weinstein SJ, Virtamo J, Hosgood HD, 3rd, et al. 2011. A prospective study of telomere length measured by monochrome multiplex quantitative PCR and risk of lung cancer. Lung Cancer 73(2): 133-137.

Shin JY, Choi YY, Jeon HS, Hwang JH, Kim SA, Kang JH, et al. 2010. Low-dose persistent organic pollutants increased telomere length in peripheral leukocytes of healthy Koreans. Mutagenesis 25(5): 511-516.

Stiller-Winkler R, Hadnagy W, Leng G, Straube E, Idel H. 1999. Immunological parameters in humans exposed to pesticides in the agricultural environment. Toxicol Lett 107(1-3): 219-224.

Svenson U, Ljungberg B, Roos G. 2009. Telomere length in peripheral blood predicts survival in clear cell renal cell carcinoma. Cancer Res 69(7): 2896-2901.

Thomas KW, Dosemeci M, Coble JB, Hoppin JA, Sheldon LS, Chapa G, et al. 2010. Assessment of a pesticide exposure intensity algorithm in the agricultural health study. J Expo Sci Environ Epidemiol 20(6): 559-569.

Tiainen A_MK, Mannisto S, Blomstedt PA, Moltchanova,Perala M.M. Kajantie E, et al., 2012. Leukocyte telomere length and its relation to food and nutrient intake in an elderly population. European Journal of Clinical Nutrition 66, 1290-1294.

Undeger U, Basaran N. 2005. Effects of pesticides on human peripheral lymphocytes in vitro: induction of DNA damage. Arch Toxicol 79(3): 169-176.

von Zglinicki T. 2002. Oxidative stress shortens telomeres. Trends Biochem Sci 27(7): 339-344.

Wentzensen IM, Mirabello L, Pfeiffer RM, Savage SA. 2011. The association of telomere length and cancer: a meta-analysis. Cancer Epidemiol Biomarkers Prev. 20(6):1238-1250.

Widmann TA, Herrmann M, Taha N, Konig J, Pfreundschuh M. 2007. Short telomeres in aggressive non-Hodgkin's lymphoma as a risk factor in lymphomagenesis. Exp Hematol 35(6): 939-946.

Table 1. Mean telomere length by selected characteristics of the cancer-free study population[a].

Characteristics	Group	n=1234	RTL Mean[c] ± SD	*P*-value
Age at buccal cell collection[b]	Q1 (41-62)	312	1.25 ± 0.34	
	Q2 (63-67)	295	1.21 ± 0.39	
	Q3 (68-72)	333	1.18 ± 0.33	
	Q4 (73-92)	294	1.18 ± 0.38	0.003
BMI	18.65-25.66)	392	1.20 ± 0.34	
	25.67-28.60)	394	1.21 ± 0.38	
	28.61-46.98)	385	1.21 ± 0.36	0.93
Applicator type	Private	1184	1.21 ± 0.36	
	Commercial	50	1.08 ± 0.43	0.01
State of residence	Iowa	831	1.19 ± 0.32	
	North Carolina	403	1.24 ± 0.43	0.03
Education	High school or lower	805	1.20 ± 0.37	
	Greater than high school	406	1.21 ± 0.36	0.71
Smoking status	Never	563	1.19 ± 0.32	
	Former	562	1.20 ± 0.38	
	Current	97	1.25 ± 0.42	0.63
Pack-years of smoking	No	563	1.19 ± 0.32	
	<20	372	1.20 ± 0.32	
	≥20	258	1.21 ± 0.47	0.90
Chewing tobacco	No	1087	1.19 ± 0.36	
	Yes	147	1.27 ± 0.34	0.01
Alcohol Drinking	No	516	1.22 ± 0.39	
	Yes	663	1.19 ± 0.31	0.10
	Low (< 3 drinks/week)	346	1.17 ± 0.34	
	High (≥ 3 dinks/week)	317	1.20 ± 0.34	0.25
Cardiovascular diseases	No	907	1.21 ± 0.36	0.88
	Yes	326	1.21 ± 0.38	
Diagnosis of Diabetes	No	1167	1.20 ± 0.36	
	Yes	66	1.20 ± 0.33	0.78
High blood pressure	No	607	1.17 ± 0.32	
	Yes	225	1.19 ± 0.33	0.22
Family history of any cancers	No	548	1.20 ± 0.33	
	Yes	613	1.24 ± 0.39	0.88
Use of any pesticides	Low (≤236 days)	582	1.21 ± 0.35	
	High (>236 days)	606	1.20 ± 0.37	0.48[1]

[a] P-values from linear regression on ln-transformed RTL (continuous) adjusted for age at buccal cell sample collection (continuous) for all characteristics other than age.

[b]Median age at buccal cell collection = 68 years.

[c]RTL mean is the arithmetic mean for the stratum-specific estimate of RTL along with standard deviations (SD) of each stratum.

Table 2. Lifetime pesticides use days, intensity-weighted lifetime pesticide use days and relative telomere length

Pesticide (class)	Pesticide Use (continous)	Lifetime Days No.	Lifetime Days RTL Mean ± SD	Lifetime Days P for trend[a] (β ± SE)	Lifetime Intensity-weighted Days No.	Lifetime Intensity-weighted Days RTL Mean ± SD	Lifetime Intensity-weighted Days P for trend[a] (β ± SE)
Herbicides							
Alachlor (chloroacetanilde)	No use	466	1.24 ± 0.41		466	1.24 ± 0.41	
	Low	225	1.18 ± 0.31		221	1.14 ± 0.28	
	Medium	215	1.18 ± 0.32		219	1.19 ± 0.36	
	High	219	1.16 ± 0.33	0.002 (-0.011 ± 0.010)	219	1.18 ± 0.32	0.005 (-0.007 ± 0.002)
Metolachlor (acetamide)	No use	622	1.23 ± 0.38		622	1.23 ± 0.38	
	Low	223	1.17 ± 0.31		165	1.18 ± 0.32	
	Medium	112	1.18 ± 0.31		164	1.18 ± 0.33	
	High	157	1.16 ± 0.37	0.01 (-0.013 ± 004)	163	1.16 ± 0.34	0.01 (-0.006 ± 0.002)
Trifluralin (dinitroaniline)	No use	515	1.23 ± 0.36		515	1.23 ± 0.36	
	Low	249	1.20 ± 0.39		200	1.17 ± 0.42	
	Medium	191	1.17 ± 0.33		199	1.19 ± 0.33	
	High	159	1.16 ± 0.33	0.05 (-0.008 ± 0.004)	199	1.18 ± 0.31	0.06 (-0.004 ± 0.002)
2,4-D (phenoxyacid)	No use	194	1.27 ± 0.48		194	1.27 ± 0.48	
	Low	366	1.21 ± 0.36		334	1.21 ± 0.37	
	Medium	320	1.21 ± 0.31		338	1.18 ± 0.32	
	High	318	1.16 ± 0.32	0.004 (-0.012 ± 0.004)	330	1.18 ± 0.31	0.02 (-0.006 ± 0.002)
Insecticide							
Dichlorodiphenyltrichloro-ethane (DDT, organochlorine)	No use	428	1.21 ± 0.34		428	1.21 ± 0.34	
	Low	153	1.13 ± 0.33		126	1.10 ± 0.33	
	Medium	97	1.13 ± 0.32		124	1.15 ± 0.30	
	High	121	1.16 ± 0.30	0.08 (-002± 0.006)	121	1.18 ± 0.31	0.03 (-0.006 ± 0.003)
Permethrin (poultry/livestock) (pyrethroid)	No use	1021	1.21 ± 0.37		1021	1.21 ± 0.37	
	Low	36	1.17 ± 0.27		34	1.16 ± 0.26	
	Medium	40	1.16 ± 0.28		35	1.12 ± 0.29	
	High	28	1.10 ± 0.26	0.02 (-0.018 ± 0.008)	33	1.14 ± 0.26	0.02 (-0.010 ± 0.004)
Toxaphene (organochlorine mixture)	No use	679	1.18 ± 0.33		679	1.18 ± 0.33	
	Low	60	1.13 ± 0.39		43	1.08 ± 0.39	
	Medium	31	1.15 ± 0.28		43	1.22 ± 0.34	
	High	37	1.14 ± 0.27	0.04 (-0.017 ± 0.009)	42	1.11 ± 0.25	0.05 (-0.008 ± 0.004)

[a]P-value of regression coefficient for ln-RTL (continuous) regressed on ln-transformed lifetime days of pesticide use (continuous) or ln-transformed lifetime intensity weighted days of pesticide use (continuous), adjusted for age at buccal collection (continuous), state (IA vs. NC), license types (private vs. commercial), use of chewing tobacco regularly for six months or longer (yes vs. no), total pesticide exposure days (continuous). RTL mean is the arithemetic mean for stratum specific RTL by tertitle of pesticide exposure, along with standard deviations (SD) of each tertile.